The Effects of Water Depth on Short-term Biofouling of Introduced Substrates.

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LONG-TERM GOALS

Our goal is to determine changes in the spectral characteristics of elastic and inelastic light scatter from substrates submerged in seawater and colonized by marine organisms. We are attempting to determine the changes in optical properties of introduced surfaces that occur with the settlement and succession of sessile organisms and their associated biofilms. We seek to develop an optical model that can predict the magnitude of fouling and the spectral characteristics of the biota and their ability to disguise man-made objects placed on the seafloor.

OBJECTIVES

The objectives of these studies are: 1) to establish the formation rate of biofilms using changes in light transmission through glass surfaces, and 2) to study fouling as a function of depth (light levels) using horizontally and vertically oriented panels of a spectrally neutral material. The primary optical property under consideration is spectral reflectance; flow cytometric enumeration of organisms, fluorescence yield and absorption are also measured on panels and fouling communities brought back to the laboratory. We expanded our measurements on the formation of biofilms to the subtropical waters off Key West, Florida, during spring and summer of this year.

APPROACH

Our approach encompassed studies in temperate and subtropical coastal waters in the Gulf of Maine and off Key West, Florida. In the Gulf of Maine, glass fouling panels were suspended below the surface for a week at a time. Discrete analysis of biofilm communities was accomplished by brushing the surface of the glass slides with a focused water jet to collect organisms in suspension. Flow cytometric analysis was used to determine the concentration of photoautotrophs keyed on pigment autofluorescence; cyanobacteria, picoplankton and cryptophytes groups were differentiated. DAPI staining was used to enumerate bacteria and microheterotrophs. Fouling as a function of depth was determined on gray PVC panel sets suspended from an optical mooring in 20m of water in the mouth

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14. ABSTRACT
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of the New Meadows River. Panel sets were fixed at the 50, 25, 10 and 5% light levels and remained at these light levels since they were tethered to surface floats. After last year's entanglements with fishing gear, the panel sets were reduced in size by half to minimize drag due to surface area. Rather than the texture experiments using glass and masonite panels attempted last year, which produced poor spectral reflectance measurements due to specular reflection and low initial reflectance of the materials, respectively, duplicate horizontal and vertical gray PVC panels were deployed to improve the likelihood of survival. In Key West, a Wet Labs C-Star transmissiometer was deployed in coastal waters to determine the high resolution time course of fouling on the glass surfaces of the optical windows. The transmissometer was deployed for 5 to 12 days and data logged every 10 minutes. Reference beam attenuation measurements were made at the beginning and end of each experiment.

WORK COMPLETED

Our observations of biofilms in the Gulf of Maine covered the period from June 12 to September 3, 2003. Three fouling experiments on glass slides were performed in surface waters off Boothbay Harbor, ME. For our studies as a function of depth, an optical mooring consisting of an internally logging Satlantic Hyperspectral reflectance buoy and battery pack was deployed in the mouth of the New Meadows River and used as a platform for the PVC fouling panel sets. Hourly reflectance data throughout the day was used to measure the background *in situ* optical properties of the environment. Diver deployed panel sets were used for two experiments and reflectance measurements of surfaces made weekly either *in situ* using a DiveSpec reflectometer (Experiment 1) or retrieved by divers for measurements aboard ship using an Analytical Spectral Devices FieldSpec reflectometer. In Key West, three deployments of a WetLabs C-Star transmissometer were performed between April and June of 2003.

RESULTS

For surface biofilm experiments, slides were retrieved daily and analyzed using a Becton Dickinson flow cytometer. Procaryotes (as phycoerythin containing cyanobacteria and DAPI stained bacteria) and eucaryotes (as chlorophyll containing picoplankton and phycoerythrin containing cryptophyes) appeared on the slides after one day and increased through the 15 day experiments (Figure 1). Photosynthetic organism concentrations increased faster than bacteria, but this result may have been affected by the harvesting method. Photosynthetic capacity ranged between 0.5 and 0.6 during the experiment indicating healthy populations of autotrophic organisms. Measurements of spectral transmission showed decreases in the blue and red regions of chlorophyll absorption and increases in the near-infrared which support the theory of photoautotrophic growth in the biofilm populations. Experiments of fouling as a function of depth were again confounded by interference by fishing gear, but a 70 day time series was obtained for vertical PVC panels at the surface as well as shorter term comparisons as a function of depth and for comparisons of vertically and horizontally oriented panels. In all cases, horizontally oriented panels fouled more quickly than vertically oriented panels, with 7 to 21 day exposures resulting in fouling rates two times higher for horizontal panels based on reflectance at selected wavelengths. Similarly, fouling rates as a function of depth over any time period based on selected wavelengths of reflectance were linearly related to PAR levels expressed as percent with the exception of the deepest panel set which was spatially light limited on the inner portion of the panel due to shading by the vertical component of the frame. Figure 2 shows the spectral reflectance of a vertical PVC surface over 70 days. Wavelengths shorter than 700nm decrease over time compared to a wetted blank panel, 701nm represents a hinge point and near-infrared wavelengths greater than 701nm increase over time. Figure 3 shows the non-linear relationships at 450, 670 and 800nm, and the hinge

point at 701nm. The continuous measurements of light attenuation by biofilms showed an induction period of 4 days and a rapid decrease to 60% after 5.5 days (Figure 4.) Visible examination of the fouling community found a heavy growth of filamentous diatoms coving the surface of the optical windows. The measurements of beam transmission are a composite of attenuation by particles in suspension and films/organisms on two glass surfaces. Measurements of beam transmission made before and after the experiment with the windows cleaned showed a 10% difference over the period, therefore, the rapid decrease after 5.5 days is logarithmic and common to the growth rates observed for photosynthetic autotrophs.

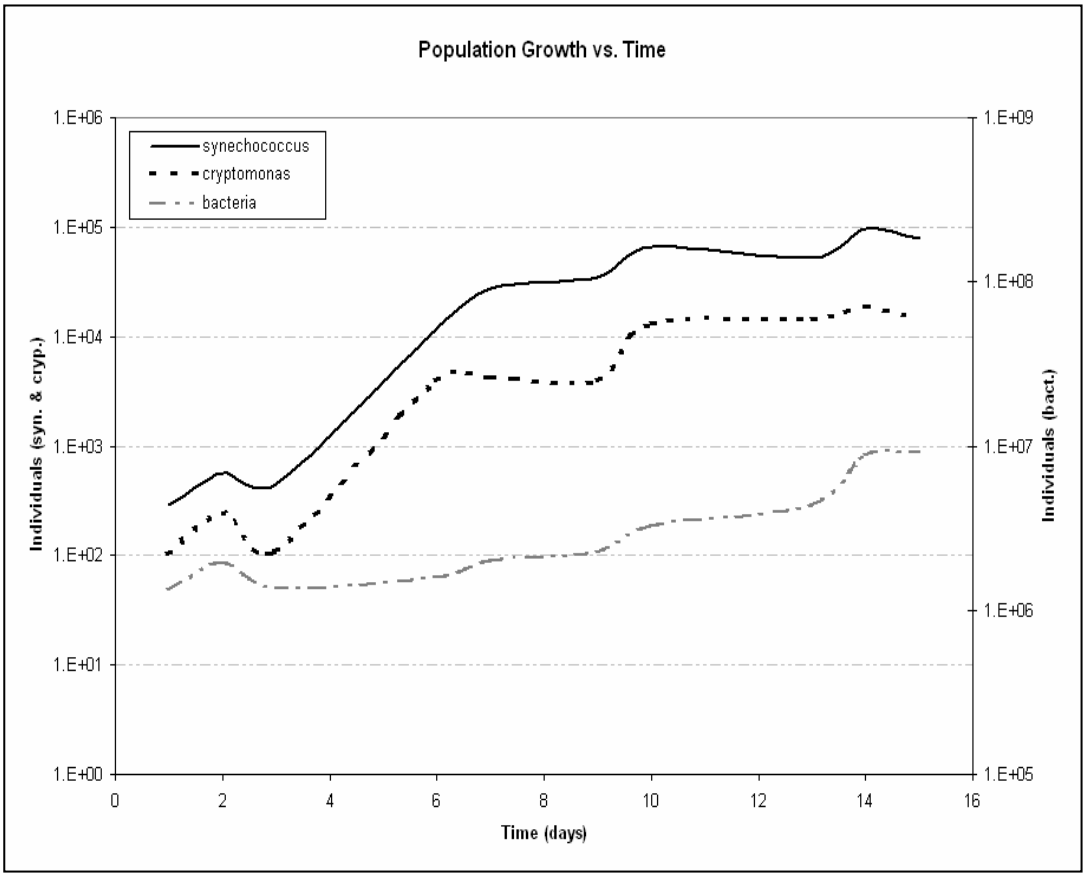

***Figure 1. Growth of microbial groups brushed from glass fouling plates as a function of time over 15 days. Bacterial growth is less than photoautotrophic organisms represented by* Synechecoccus *and* Cryptomonas.**

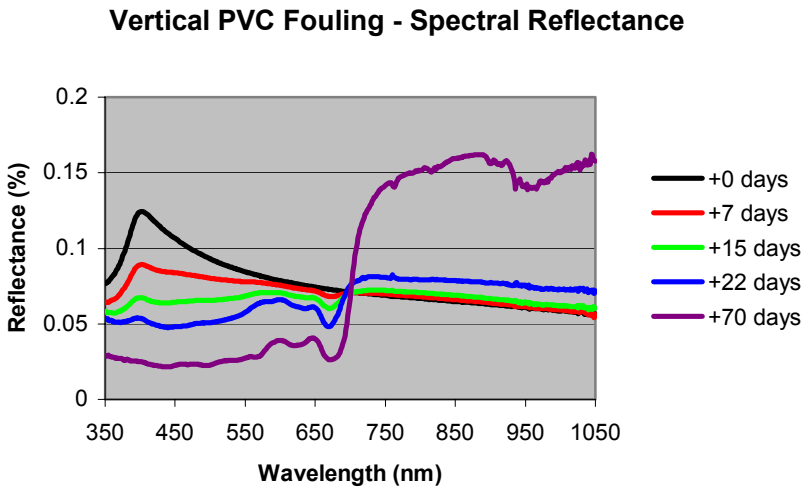

Figure 2. Spectral reflectance of a vertical PVC surface over 70 days. Wavelengths shorter than 700nm decreasedover time due to photosynthetic pigment absorption, 701nm is the hinge point where no change occurs over time and near-infrared reflectance increases over time due to the presence of plant biomass.

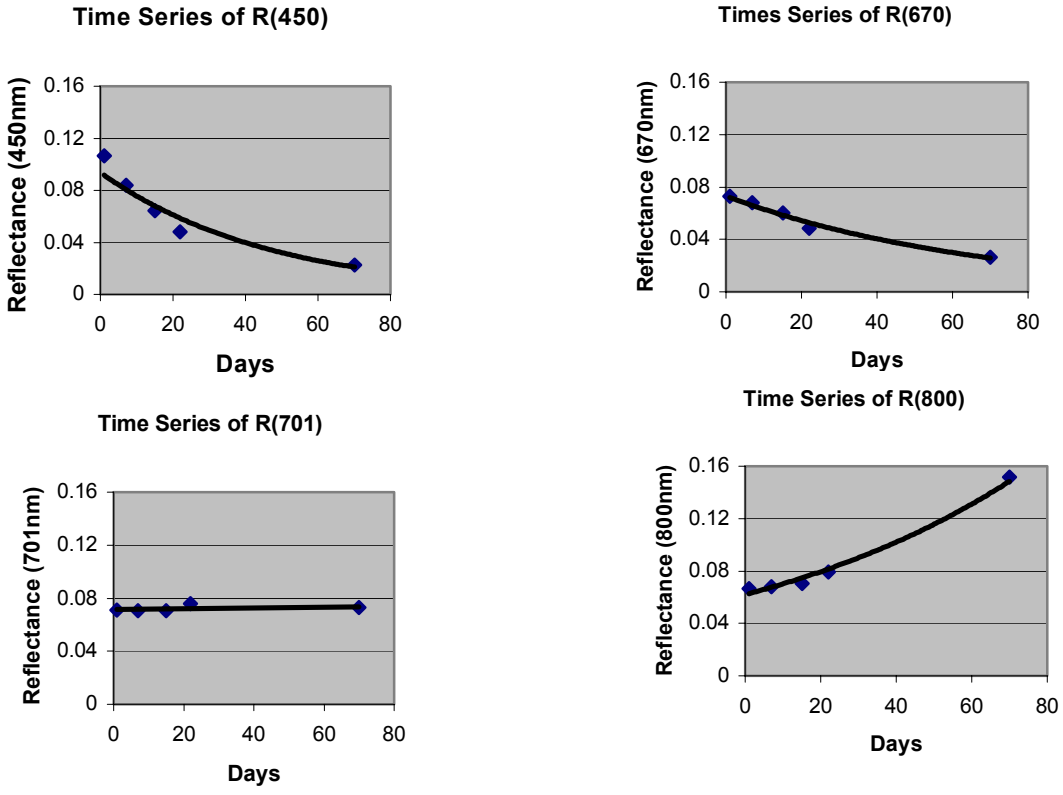

Figure 3. Curve fits to selected wavelengths of reflectance as a function of time. Reflectance at 450 and 670nm show negative non-linear trends, 701nm is the hinge point where reflectance remains constant and 800nm shows a positive non-linear trend indicative of the near-IR region.

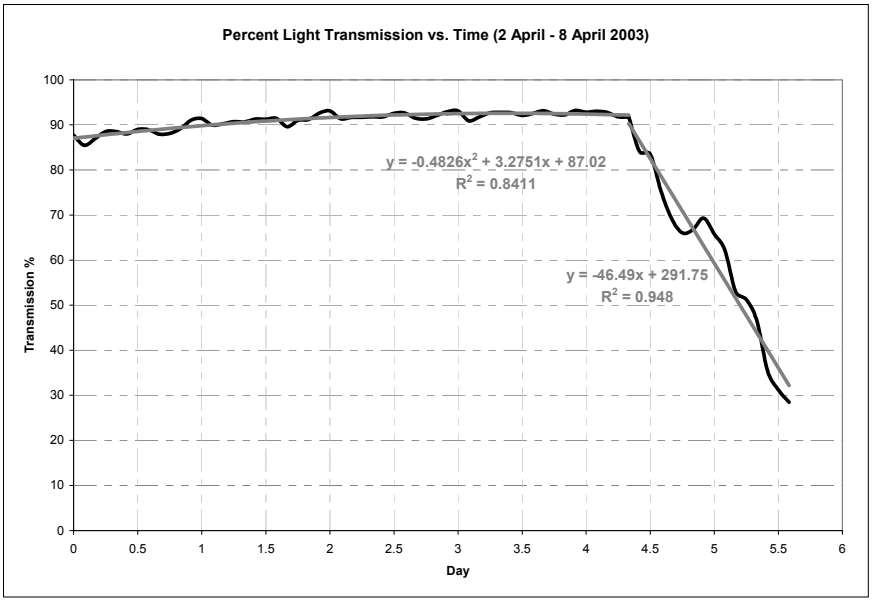

Figure 4. Beam transmissometer data for 5.5 day fouling experiment off Key West, FL. Dark line is % transmission measured every 10 minutes, gray line is curve fit to the data. Beam transmission remained relatively constant for 4 days, then decreased by 60% over the next 1.5 days.

IMPACT/APPLICATIONS

The potential impact of this area of research is much broader than we would have imagined, leading us into related projects concerned with fresh water generating systems applications, materials science and ballast water treatment. While the notion of fouling communities mitigating surface charge or ionic transitions in materials submerged in seawater may not be new, recent advances in technology and materials may result in renewed interest by the Navy in these research areas.

RELATED PROJECTS

Our continued collaboration with Dr. Mike Sieracki and colleagues at the Center for Flow and Imaging Cytometry at Bigelow Laboratory for Ocean Sciences has benefited our results of microbial growth on glass fouling plates. Dr. Kelly Rankin from Stevens Institute of Technology has assisted with experiments and contributed expertise in particle resuspension. Dr. Charles Mazel of Psicorp, Inc., has assisted with measurements of *in situ* reflectance using the DiveSpec. This work has connected to studies of the application of reverse osmosis systems aboard naval vessels in coastal waters through Mr. Ted Lemieux, Director of the Corrosion Research Facility at NRL, Key West, and in studies of materials enoblement through Dr. Farrell Martin at NRL, Washington, DC.

PUBLICATIONS

Schwarz, J., P. Kowalczuk, S. Kaczmarek, G.F. Cota, B.G. Mitchell, F.P. Chavez, A. Cunningham, D. McKee, P. Gege, M. Kishino, D.A. Phinney and R. Raine. 2002. Two models for absorption by coloured dissolved organic matter (CDOM). Oceanologia, 44: 209-241. [published, refereed]

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